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Field Evaluation of the Effectiveness of an Oral Toltrazuril and Iron Combination (Baycox® Iron) in Maintaining Weaning Weight by Preventing Coccidiosis and Anaemia in Neonatal Piglets

Kristina Streyl[1], Janaina Carlstron[2], Eliana Dantos[2], Roberto Mendoza[3], Juan Agustin Torres Islas[4], Chandra Bhushan[1]

[1] Bayer Animal Health GmbH, 51368 Leverkusen, Germany
[2] Bayer Animal Health, Sao Paulo, Brazil
[3] Asesoría Integral Negocio Porcino, Querétaro, Mexico
[4] Bayer Mexico S.A., Granada, Mexico

Corresponding author:
Chandra Bhushan
E-mail: chandra.bhushan@bayer.com

Abstract

Effectiveness of an oral combination of toltrazuril and iron dextran (Baycox® Iron) to maintain weaning weight by preventing coccidiosis caused by *Isospora suis* and iron-deficiency anaemia in neonatal piglets was investigated on three commercial pig farms with a history of coccidiosis: two in Mexico and one in Brazil. On day (SD) 2 of life, piglets were randomised within litter by bodyweight to treatment or control group. On SD 3 piglets allocated to the control group (CG) each received 1 mL Baycox®, containing 50 mg/mL toltrazuril orally and commercially available iron (200 mg/piglet) by intramuscular injection. Piglets allocated to the treatment group (TG) each received 1 mL toltrazuril and iron combination orally (Baycox® Iron) containing 50 mg/mL toltrazuril and 228 mg iron as iron dextran. All piglets had access to creep feed. 6493 piglets completed the study. Bodyweight at weaning on SD 21 of piglets treated with the oral toltrazuril and iron combination was confirmed to be non-inferior to the control treatment with <1% difference between group mean body weights. Faecal samples from at least 10% of litters on SD 14 demonstrated control of coccidiosis. Haemoglobin levels on SD 21 were lower in the oral toltrazuril and iron combination treated piglets compared to control levels but above minimum haemoglobin levels to maintain health. There was no difference in mortality between the two groups. This large scale field evaluation clearly demonstrated the effectiveness of a combination of oral toltrazuril and iron (Baycox® Iron) in maintaining body weight at weaning compared

to conventional treatment. The combination was effective in preventing coccidiosis and anaemia and thus provides a valuable alternative that reduces stressful events in neonatal piglets. There were no product related adverse events.

Introduction

Coccidiosis in piglets is generally caused by the single species *Isospora suis* (Mundt 2005). It is an important cause of morbidity in the neonatal piglet, capable of causing disease during the first days of life, at the same time as the piglet undergoes physiological changes following birth and is growing rapidly. It has been identified as the most common cause of diarrhoea in the neonatal pig (Driesen et al. 1993). Following ingestion of sporulated oocysts from the environment, infection develops over 5–7 days and results in damage to the villous epithelium, sloughing of cells, crypt fusion and ultimately villous atrophy. The worst damage is associated with the asexual stage which means that diarrhoea can appear 1–2 days prior to oocysts appearing in the faeces. The age of infection (younger piglets tend to be more severely affected) and the level of infection (higher oocyst burdens result in more severe infections) have a bearing on the overall severity of infection (Mundt et al. 2003). Oocysts are then passed in the faeces in large numbers for approximately 5–16 days. Typically disease occurs in young, suckling pigs aged approximately 5–14 days of age. Affected pigs pass yellow to grey, pasty diarrhoea, show reduced weight gain or weight loss and may become hairy. There is considerable morbidity of affected individuals but mortality is generally low. Infection with *I. suis* may be complicated by concomitant bacterial or viral infections (Chae et al. 1998). Immunity to *I. suis* infection appears to be age-related and associated with non-specific immunity to a large extent, hence prevention of the effects of infection are necessarily focussed on neonatal piglets (Driesen et al. 1993; Mundt et al. 2003). It is difficult to diagnose the infection in the pre-patent stage other than by history on the farm, the presence of diarrhoea and pathological evidence in the intestine at post-mortem examination.

Coccidiosis occurs worldwide in association with intensive pig husbandry. For example, infection is prevalent in Canada, with 70 % of farms affected (Sanford and Josephson 1981, Aliaga-Leyton et al. 2011). The prevalence of *I. suis* in grown pigs was 5.7 % in a survey conducted in Brazil (Da Silva et al. 1990). In Europe, a 2003 survey involving approximately 3,500 litters on over 400 farms in 12 countries showed that *I. suis* was widespread in all countries; overall 69 % of the farms and 25 % of litters on those farms surveyed were shown to be positive (Mundt et al. 2005). In The Netherlands, the litter prevalence was 53 % on farms where *I. suis* had been identified (Eysker et al. 1994). In Australia, *I. suis* was present in 53.8 % of samples from over 2,300 piglets and in over 70 % of the 151 piggeries surveyed (Driesen et al. 1993). In Korea 17.3 % of piglets from 304 farms were reported as positive for *I. suis* (Chae et al. 1998).

During the 20th Century it was recognised that the move to house pigs indoors for intensive production, which prevents piglets having access to earth, results in iron deficiency anaemia unless piglets are provided with iron supplementation. Although piglets are born with iron in reserve (Venn et al. 1947), their growth requirement outstrips their reserve by the time they are a week to ten days of age, since sow's milk supplies only about 1 mg of a required 7 mg per day (McDonald et al. 1988). A lack of sufficient iron results in iron deficiency anaemia, with pallor as the most obvious sign, and may result in reduced feed intake and growth rates. Normal reference ranges for adult pigs are 9-13 g/dL of haemoglobin and it is generally accepted that a piglet is borderline anaemic when haemoglobin levels are below 8 g/dL with severe anaemia at 6 g/dL (Kegley et al 2002). Post-weaning, the diet normally provides sufficient iron, so anaemia is mainly seen in the suckling piglet unless iron is supplemented.

Traditional prevention of coccidiosis and anaemia has involved two separate interventions. Coccidiosis control is commonly achieved with the administration of anticoccidials in the pre-patent period (day 3–5 of life) and toltrazuril is well established as an effective anticoccidial. For example, toltrazuril administered at a dose of 20 30 mg/kg to piglets at 3 days of age provided excellent prophylaxis against *I. suis* coccidiosis with significantly less diarrhoea and lower oocyst counts at approximately 12 and 21 days of age when compared to an untreated control group (Madsen et al. 1994). A single oral treatment with toltrazuril administered during the pre-patent period provided effective and sustained suppression of oocyst shedding and diarrhoea in piglets experimentally infected with *I. suis* (Joachim and Mundt 2011). In an on-farm Venezuelan study (Boulanger et al. 1994), where piglets were treated at 5 days of age with 20 mg/kg toltrazuril, treated piglets were heavier at weaning and had lower mortality than their untreated counterparts. There is evidence that treatment with toltrazuril does not interfere with the acquisition of immunity to coccidial infection (Greif 2000).

Traditionally iron supplementation is administered to piglets when they are a few days of age by intramuscular injection of iron dextran (Lipiński et al. 2010). However it is recognised that intramuscular injection can be associated with transfer of infection through contaminated needles and is a relatively time-consuming process with an inevitable degree of stress involved for the piglets. Oral administration has been shown to be an effective alternative (Maes et al. 2011), relying on adequate absorption through the duodenum for the supplementation to be beneficial.

During their first week of life piglets may undergo a series of manual interventions that aim to ensure that piglets and sows remain healthy such as iron injection, tail docking and castration, and on farms with a history of coccidiosis, anti-coccidial metaphylaxis. If it is possible to provide a less invasive alternative to iron injection that occurs simultaneously with anti-coccidial treatment on farms with history of coccidiosis, and thereby reduces the total number of interventions, this provides a potential welfare benefit for the piglet and labour-saving gain for the farmer.

Today's commercial pig production is typically an industry with a narrow profit margin (Plain and Lawrence 2003). After feed, labour is the highest cost input of production (Plain and Lawrence 2003), thus time-saving interventions are likely to be beneficial, however such interventions cannot compromise productivity, which is largely determined by health.

This paper presents the results of studies conducted on three farms in Mexico and Brazil examining the effectiveness of an oral toltrazuril and iron combination (Baycox® Iron) in maintaining weaning weight by preventing coccidiosis and iron deficiency anaemia in newborn piglets. The oral toltrazuril and iron combination is based on Baycox® 5 % suspension which has been authorised for use in many parts of the world since 1998, containing 50 mg/mL toltrazuril with the addition of 228 mg/mL (22.8 % w/v) oral iron as iron dextran, designed to provide pig producers with a safe, effective and convenient method of preventing coccidiosis combined with iron supplementation.

Materials and methods

Farms

Three commercial pig farms, two in Mexico and one in Brazil, with a history of coccidiosis were included in the study which was conducted from September to December 2014. All farms offered creep feed to piglets as part of their standard management practice.

Piglets

All piglets within a litter were weighed and ranked according to body weight on SD 2. Healthy piglets weighing 900g or more were blocked by body weight and randomly allocated to treatment or control groups. Piglets were weighed for a second time on SD 21. A total of 7057 piglets were enrolled into the study of which 6475 completed the study, had complete data sets and were included in the analysis.

Treatments

On SD 3 piglets allocated to the control group (CG) each received 1 mL Baycox®, containing 50mg/mL toltrazuril orally and commercially available iron (200 mg/piglet) by intramuscular injection. Piglets allocated to the treatment group (TG) each received 1 mL toltrazuril and iron combination orally (Baycox® Iron) containing 50 mg/mL toltrazuril and 228 mg iron as iron dextran.

Faecal samples and coccidial oocyst counts

Faecal samples were collected from at least 10 % of litters on SD 14±1. Each sample was scored between 1–4 with 1 being normal, 2 pasty, 3 semi-liquid and 4 liquid and examined for coccidial oocysts using light microscopy.

Haemoglobin

Within each litter, two mid-weight piglets, one from TG and one from CG, were identified and blood samples were collected from each of these piglets on SD 3 (prior to treatment) and SD 21 for haemoglobin measurement. Blood samples were analysed by two local laboratories, one in Mexico for samples from both Mexican farms and a second one in Brazil using their standard methods.

Deaths and piglets euthanased

The date of death was recorded for any piglets that were found dead or had to be euthanased during the study.

Statistical analysis

Data from piglets which had died or where there were incomplete records were excluded from the analysis of body weight and haemoglobin.

Initial body weights were analysed for farm and treatment effects using ANOVA methods, to check the efficiency of group allocation. The significance level was set at 5 %.

The primary criterion for evaluating performance was body weight at weaning. The study was designed as a non-inferiority study. The null hypothesis of this non-inferiority test was that the mean piglet weight in TG was worse than in the CG by more than 120 g. The alternative hypothesis was that the TG was no worse than CG. The bilateral 95 % confidence interval around the mean difference was constructed using the residual variance from the Analysis of Variance table, based on the formula:

95 % Confidence Interval = Mean (treatment) difference

Oocyst excretion, faecal consistency and mortality were considered as secondary criteria. Faecal consistency was scored from 1–4 with 1 being firm and 4 liquid.
Haemoglobin levels at beginning of the study and at weaning were analysed for farm and treatment effects using ANOVA with repeated design, the significance level was set at 5 %.

The number of piglets dead or missing at Day 21 was compared between groups using a chi-square test. All calculations were conducted using Minitab version 17, except the confidence interval calculations for non-inferiority, where an Excel spreadsheet was used.
Interpretation was assisted, where appropriate, with the use of boxplots.

Results

A total of 7057 piglets were enrolled and a complete dataset was available for 6493 which were included in the analysis. By farm, 1194 piglets from Farm 1 (Mexico), 2217 from Farm 2 (Mexico) and 3082 from Farm 3 (Brazil) were included in the analysis.

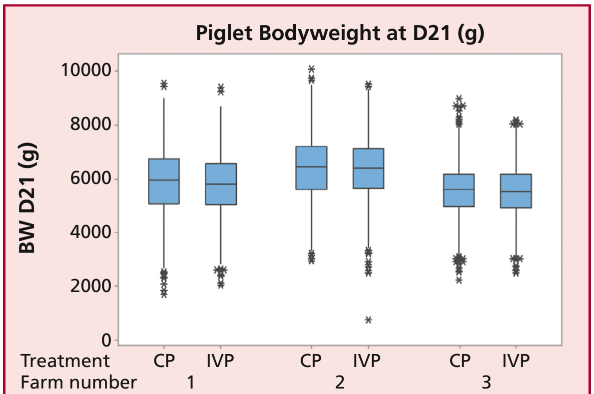

Fig. 1 Boxplot of Body Weight on SD 2 by Farm and by Group; CP: control group; IVP: treatment group; bw: body weight

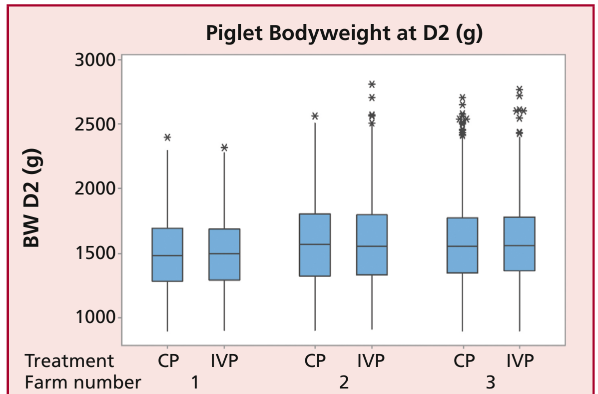

Fig. 2 Boxplot of Body Weight on SD 21 by Farm and by Group; CP: control group; IVP: treatment group; bw: body weight

The mean piglet body weight on SD 2 was 1.56 kg for the TG piglets and 1.56 kg for the CG piglets (Table 1 and Figure 1). Analysis of variance showed no significant difference between treatment group body weights on SD 2 (p=0.6). A significant farm effect was observed, with a maximum difference of 80 g in mean CG piglet body weights between piglets in Farms 1 and 2, compared with a 70 g difference in mean CG bodyweight between Farms 1 and 3.

On SD 21 the mean TG piglet weight by farm varied between 5.52 and 6.36 kg with an overall mean of 5.85 kg, whilst CG piglets weighed between 5.57 and 6.40 kg by farm with an overall mean of 5.90 kg (Table 2 and Figure 2). On SD 21, analysis of variance conducted using body weight at SD 2 as a covariate showed a significant farm effect with piglets in Farm 2 heavier than those in Farm 1, and piglets in Farm 3 were lightest, on average. The smallest difference between farm mean CG body weights on SD 21 was 250 g (Farm 3 and Farm 1) and the largest 830 g (Farm 2 and Farm 3). There was a significant association between body weight on SD 2 with body weight on SD 21 as piglets that were heavier on SD 2 more likely to be heavier at weaning. There was a 50 g weaning mean weight difference between piglets in the two treatment groups, which equated to a 0.84 % difference between the two groups. The result was confirmed to be non-inferior because the confidence interval of the mean (-100.8 g to -1.5 g) was totally contained within the previously selected non-inferiority margin of 120 g.

Coccidial oocyst counts on all farms were low indicating that toltrazuril treatment controlled coccidial infection, with faecal scores <3 in all except 15 samples which were similarly distributed between TG and CG piglets.

Mean farm haemoglobin levels on SD 3 ranged from 8.58 – 10.48 g/dL with an overall mean of 9.55 g/dL for the TG and 9.63 g/dL for the CG (Table 3). ANOVA showed a significant farm effect, with haemoglobin levels notably higher in piglets from Farm 3, but no treatment group effect (p=0.705). The mean TG haemoglobin level on SD 21 showed an increase of 0.32 g/dL over the mean level in the same group on SD 3. On SD 21 the overall mean haemoglobin concentration for TG piglets was 9.87 g/dL compared to a mean of 11.53 g/dL for the CG piglets (Table 4). ANOVA on SD 21 showed a treatment-related effect (p =0.000). In addition, a farm effect was observed (p=0.000), with piglets in Farm 1 having the lowest levels, in Farm 2 the highest, and in Farm 3 intermediate). A farm*treatment interaction was also detected (p=0.000), indicating that treatment had a different effect on haemoglobin levels according to the farm.

Table 1 Mean Piglet Body weights on SD 2 by Group and by Farm (kg)

	Treatment Group			Control Group		
	n	Mean	St dev	n	Mean	St dev
Farm 1	611	1.50	0.28	610	1.50	0.29
Farm 2	1167	1.58	0.32	1165	1.58	0.32
Farm 3	1752	1.58	0.31	1752	1.57	0.31
Overall	**3530**	**1.56**	**0.31**	**3527**	**1.56**	**0.31**

n: number; st dev: standard deviation

Table 2 Mean Piglet Body weights on SD 21 by Group and by Farm (kg)

	Treatment Group			Control Group		
	n[a]	Mean	St dev	n[a]	Mean	St dev
Farm 1	595	5.73	1.18	599	5.82	1.25
Farm 2	1114	6.36	1.15	1103	6.40	1.17
Farm 3	1533	5.52	0.98	1549	5.57	1.03
Overall	**3242**	**5.85**	**1.14**	**3251**	**5.90**	**1.18**

[a]: includes only piglets with a full dataset included in the analysis; n: number; st dev: standard deviation

Table 3 Mean Haemoglobin Levels on SD 3 by Group and by Farm (g/dL)

	Treatment Group			Control Group		
	n[a]	Mean	St dev	n[a]	Mean	St dev
Farm 1	99	8.84	1.41	98	8.84	1.50
Farm 2	160	8.63	1.30	156	8.58	1.35
Farm 3	286	10.32	1.69	286	10.48	1.71
Overall	**545**	**9.55**	**1.73**	**540**	**9.63**	**1.81**

[a]: includes only piglets with a full dataset included in the analysis; n: number; st dev: standard deviation

Table 4 Mean Haemoglobin levels on SD 21 by Group and by Farm (g/dL)

	Investigational Product				Control Product		
	n[a]	Mean	St dev		n[a]	Mean	St dev
Farm 1	99	8.58	1.80		98	9.58	1.16
Farm 2	160	9.89	1.73		156	12.1	1.06
Farm 3	286	10.32	1.58		286	11.91	1.26
Overall	**545**	**9.87**	**1.78**		**540**	**11.53**	**1.50**

[a]: includes only piglets with a full dataset included in the analysis; n: number; st dev: standard deviation

Consolidated mortality and missing piglet data were analysed using a Chi-Square test. No significant association between number of piglets classified as dead or missing on SD 21 (where missing was defined as missing body weights and presumed dead) and treatment group was detected ($p = 0.362$). The combination product was safe without product related adverse events.

Discussion

By having individual piglets within litters allocated to treatment or control groups, the design of this study overcame the between-litter variation that may result when treatment is allocated to all piglets within a litter. Analysis of SD 2 body weights indicated that there was no significant difference between groups on SD 2, thus confirming the randomisation process resulted in two similar groups. There was a significant difference in mean body weight between farms, illustrating the difference that farm management practices can have.

By weaning on SD 21 piglets in both groups had reached expected weaning weights and the minor difference of 50g fell within the pre-defined non-inferiority boundaries and hence significant non-inferiority was established. The mean difference between treatment groups was smaller than the smallest difference in CG mean weights between farms. Replacement of an intramuscular iron injection with oral iron and combining this with anticoccidial toltrazuril treatment provides an animal welfare improvement without reduction in productivity.

The mean TG haemoglobin levels on SD 3 were slightly, although not significantly, less than those of the CG. There was no evidence that the lower haemoglobin levels in the TG compared to the CG on SD 21 resulted in clinical signs of anaemia or greater morbidity or mortality than that of CG piglets. Thus the convenience and reduced stress benefits of toltrazuril combined with iron in an oral formulation did not result in inferior performance and provide an excellent alternative to conventional treatment with oral toltrazuril and iron administered by intramuscular injection.

Conclusion

The present data generated in a large scale field evaluation clearly demonstrated the effectiveness of a combination of oral toltrazuril and iron combination (Baycox® Iron) in maintaining body weight at weaning compared to conventional oral toltrazuril and iron by injection. The combination was effective in preventing coccidiosis and iron deficiency anaemia in neonatal piglets. It thus provides a convenient, labour-saving and valuable alternative that reduces stressful events in piglets in their first week of life.

Ethical standards

All national guidelines for the care of animals were followed.

Funding

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Conflict of interest

Roberto Mendoza is employed by Asesoría Integral Negocio Porcino, all other authors are employed by Bayer Animal Health.

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